

## Comparison of Soleus Muscle Fibers in Rats and Rabbits at Different Stages of Postnatal Development

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**Abstract:** In the present study, the effects of postnatal development on the number and distribution of skeletal muscle fibers of different types in hind leg of rat and rabbit were studied. The soleus muscles of rats aged 2 days, 2, 4, 6, 8, and 10 weeks (body weight 10, 32, 95, 190, 280, and 320 g), and rabbits aged 2 days, 2, 4, 8, 12, 16, 20, and 24 weeks (body weight 100, 220, 400, 750, 1 200, 1 600, 2 100, and 2 500 g) were stained with succinic dehydrogenase. With an image analysis system, the X-Y coordinates of fibers were used to analyze the growth-related changes. The results of present study showed that three types of fibers were found in the soleus muscles of rat and rabbit, i.e., type I (slow oxidative), II X (fast oxidative), and II A (fast oxidative glycolytic). The type I fibers were present throughout the muscle that had a uniform distribution and tended to increase in number with aging. Type II X fibers were scattered throughout the muscle and decreased markedly in number with aging. Type II A fibers were located at the central and deep regions, and showed a little or no change in number and distribution with aging. While be of age, type II A and II X fibers became restricted to the superficial region. No type II B fibers were detected. Type II A fibers had the largest diameter, type I intermediate and type II X the smallest. Mean cross-sectional area of each type fibers of rabbits was larger than that of rats. The present results indicate that the number and distribution of muscle fibers of different types in hind limb of rat and rabbit change with the process of postnatal growth.

**Key words:** Rat; Rabbit; Muscle fiber type; Postnatal development

## 大鼠和家兔生后发育各阶段比目鱼肌纤维的比较

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**摘要:** 为研究大鼠与家兔骨骼肌各类型肌纤维的数量和二维分布以及生后发育对其影响, 取生后 2 d 和 2、4、6、8、10 周龄 (体重 10 g 和 32、95、190、280、320 g) 大鼠及生后 2 d 和 2、4、8、12、16、20、24 周龄 (体重 100 g 和 220、400、750、1 200、1 600、2 100、2 500 g) 家兔的比目鱼肌做琥珀酸脱氢酶染色。实验结果表明, 大鼠和家兔比目鱼肌纤维被分成 I 型 (SO), II X 型 (FO) 和 II A 型 (FOG) 3 型。使用图像分析系统分析每型肌纤维在生后发育各阶段的相关变化, 大鼠和家兔比目鱼肌中: I 型纤维分布于整块肌肉, 其数量随着生后发育而增加。幼体 II X 型纤维分布在整块肌肉中, 其数量随生后发育而减少; II A 型分布在肌肉中深层, 数量几乎无变化; 至成体时只有少量的 II X 和 II A 分布在肌表层。整个发育期间未见 II B 型纤维。II A 型纤维直径最大, I 型中等, 而 II X 型最小。家兔 3 型肌纤维的平均横切面积比大鼠的大。这些结果表明大鼠和家兔后肢肌各种类型肌纤维的数量比例和分布随生长过程发生改变。

**关键词:** 大鼠; 家兔; 肌纤维型; 发育

**中图分类号:** Q954.66 **文献标识码:** A **文章编号:** 0254–5853(2004)06–0543–08

Mammalian skeletal muscles are classified into red and white muscles. The red muscle mainly comprises

Received date: 2004–05–28; Accepted date: 2004–09–10

Foundation item: This project was supported by funding from the Education Department of Jiangsu Province

\* Original Nantong Teachers' College (原南通师范学院)

收稿日期: 2004–05–28; 接受日期: 2004–09–10

基金项目: 江苏省教育厅资助项目

type I fibers that show slow-twitch oxidative, fatigue resistant properties. On the other hand, the white muscle comprises type II fibers that show fast-twitch glycolytic, fatigue properties (Guth & Samaha, 1969). Type II fibers are further divided into II A and II B subtypes based on their succinic dehydrogenase (SDHase) activity (Reichmann & Pette, 1982; Zhu, 2003a, b) and myofibrillar adenosine triphosphatase (mATPase) activity (Tunell & Hart, 1977; Hennig & Lomo, 1985). Based on their metabolic and contractile properties, type I, II A, and II B fibers in many species are corresponded to slow-twitch oxidative (SO), fast-twitch oxidative glycolytic (FOG), fast-twitch glycolytic (FG), respectively (Peter et al, 1972; Zhu et al, 2002). In the normal rat and rabbit, type I, II X/II D, II A and II B could be identified (Gorza, 1990; Lexell et al, 1994) and seem to be closely related to SO, FO (fast-twitch oxidative), FOG and FG respectively (Graziotti et al, 2001). The oxidative enzyme content of type II X/II D was confirmed as intermediate type between I and II A fibers (Pellegrino et al, 2003). We expressed type II X/II D as type II X in the present study.

The fibers of four types are distributed heterogeneously in skeletal muscles (Lefaucheur et al, 2002). The number of fibers of each type in any skeletal muscle could change with maturation, aging, exercise and different pathological conditions (Ozaki et al, 2001; Gojo et al, 2002; Russell et al, 2003; D'Antona et al, 2003) and with surgical operations (Beitzel et al, 2004; Casar et al, 2004). Thus, slow muscle fibers might transfer to predominantly fast muscle fibers. The percentage of fibers of each type in the muscle changes in accordance with the physiological properties such as contractile time, maximum shortening velocity, and fatigability. The soleus muscle is known as slow or red muscle. Kanda & Hashizume (1989) reported that the percentage of slow-twitch fibers increased with aging. However, some workers reported that the percentage of fast-twitch fibers increased with aging (Silbermann et al, 1983). In addition, Frontera et al (2000) reported a little change in the percentage of fibers of different types with aging. Pette & Staron (2000) indicated that aging was associated with a shift in type II B to II A to II X and to I. Even in the same muscle of the same age, the percentage of fibers of different types varied with exercise and pathological conditions (Bodine et

al, 2001).

The different results by investigators caused other researchers to examine the spatial distribution of muscle fibers of different types in a limited small area of the muscle cross-section (Staron et al, 1999; Mattson et al, 2002). The purpose of the present study was to investigate the growth-related changes in the number and spatial distribution of fibers of major types in whole muscle.

## 1 Materials and Methods

### 1.1 Experimental animals

Male Sprague-Dawley rats aged 2 days, 2, 4, 6, 8, and 10 (adult) weeks (body weight 10, 32, 95, 190, 280, and 320 g); Male Chinese domestic rabbits aged 2 days, 2, 4, 8, 12, 16, 20, and 24 (adult) weeks (body weight 100, 220, 400, 750, 1 200, 1 600, 2 100, and 2 500 g); six animals of each age group were used in the present study.

Rats were anaesthetized with an intraperitoneal (i.p.) injection of compound anaesthetic (0.1 mL/100 g body mass) and rabbits were anaesthetized with an intravenous (i.v.) injection of 25% urethane (1 g/kg body mass). The soleus muscles were dissected bilaterally and cut from their tendons. After measuring the muscle length, each muscle was immersed in OCT compound (Tissue-Tek, Tokyo, Japan) and frozen in isopentane precooled in liquid nitrogen ( $-196^{\circ}\text{C}$ ). The frozen muscles were stored at  $-80^{\circ}\text{C}$  overnight. Three 10  $\mu\text{m}$  thick cross-sections and one 10  $\mu\text{m}$  thick vertical-sections were cut serially at the mid-point level of the muscle length using a cryostat (Leica CM1800, Germany) at  $-20^{\circ}\text{C}$ . The first cross-section and the second vertical-section were for SDHase (Chen & Zhu, 2003). Two of the four consecutive sections were preincubated in a reaction solution for mATPase (pH 4.35 and 10.30) and stained with ammonium sulfide solution (Zhu & Zhang, 1994). Sections were cut and stained on the same day to avoid any possible loss of enzyme activity.

### 1.2 Enzyme histochemistry

The rat and rabbit tissues were respectively stained by the following histochemical methods: mATPase after acid (pH 4.35) and alkaline (pH 10.30) preincubation according to Zhu & Zhang (1994) and SDHase according to Chen & Zhu (2003). The staining pattern of I, II X, II A and II B fibers after the SDHase histochemical reaction modified by Chen &

Zhu (2003) is demonstrated on the basis of the color contract of nitro dark blue. Other intermediate types between type I, II X, II A and II B were classified into four with reference to the slides treated with mATPase and ammonium sulfide solution.

For muscle fiber counting all reacted sections were examined by light microscopy (Axiomat, Zeiss, Oberkochen, Germany) at a  $\times 100$  magnification and the resulting random fields were displayed on the monitor of a personal computer by means of a video camera attached to the microscope. The histomorphometric measurements and counts were performed with a pen linked to the personal computer using a semiautomatic image analysis system (LUCIAM, Nikon Laboratory Imaging, Prague, Czech Republic). To minimise the occurrence of operator-dependent errors the cross sections were analysed by 3 experienced investigators. More than 300 muscle fibers in 3 random fields were counted in each section. After comparison of the serial sections stained with SDHase and mATPase under acid (pH 4.35) and alkaline (pH 10.30) preincubation, the muscle fibers were classified into types I and II. Differentiation into type II X/II D fast twitch oxidative fibers, type II A fast twitch oxidative glycolytic fibers, type II B fast twitch glycolytic fibers, was performed on sections stained for SDHase activity.

## 2 Results

### 2.1 Staining with SDHase and metachromatic dye

Based on staining with metachromatic dye, three fiber types of rat and rabbit soleus muscles during different stages of postnatal development were identified on the same slide except type II B (Figs. 1 - 28). Type I showed diffuse oxidative enzyme activity or blue, II X coarse network distributed in subsarcolemmal regions or darker blue, and II A characteristic subsar-

colemmal accumulations of reaction product or light blue.

### 2.2 Distribution and cross-sectional areas of fibers of different types

In the soleus muscles of the rats and rabbits (Table 1), type I, II X and II A were present, but type II B could not be detected in any age. The soleus muscles were composed predominantly of type I with some type II A and a few type II X fibers in all ages except postnatal 2 days. The number of type I fibers increased gradually and had uniform distribution that there was less regional variation with ageing. On the other hand, type II A fibers were located at the central and deep regions in rats aged 4 to 6 weeks and rabbits aged 4 to 12 weeks. Both the number and distribution of type II A fibers showed a little or no change in rats aged 8 to 10 weeks and rabbits aged 16 to 24 weeks. A few type II X fibers were scattered throughout the muscle of rats aged 2 to 6 weeks and rabbits aged 2 to 8 weeks, and decreased in number markedly in both rats and rabbits with aging. In elderly rats and rabbits, type I fibers were dominant in the soleus muscle while only a few type II A and II X fibers were present and became restricted to the superficial region with growth.

The morphometric analysis of the muscle fibers indicated the fiber type and fiber size were closely correlated to the postnatal development of rats and rabbits (Table 2). In addition, the same morphometric of mosaic patterns of three types were observed in all muscles. The type II A fibers were found to have the largest diameter, type I intermediate and type II X the smallest. The mean cross-sectional areas of fibers of three types in rats and rabbits increased markedly with postnatal growth. Mean cross-sectional area of fibers of each type in rabbits was larger than that in rats.

**Table 1 Fiber type composition in soleus muscle of rats and rabbits at postnatal development** (Mean  $\pm$  SD, %)

Rat							Rabbit						
Age (week)	Left side			Right side			Age (week)	Left side			Right side		
	I	II X	II A	I	II X	II A		I	II X	II A	I	II X	II A
2 (d)	0	0	0	0	0	0	2 (d)	0	0	0	0	0	0
2	61 ± 5	12 ± 3	27 ± 4	62 ± 6	10 ± 2	28 ± 4	2	53 ± 8	17 ± 2	30 ± 3	53 ± 5	19 ± 3	28 ± 3
4	64 ± 4	11 ± 2	25 ± 3	64 ± 6	9 ± 2	27 ± 3	4	54 ± 5	14 ± 3	32 ± 4	54 ± 3	18 ± 2	28 ± 2
6	65 ± 3	11 ± 2	24 ± 4	66 ± 4	9 ± 3	25 ± 6	8	56 ± 3	14 ± 1	30 ± 3	57 ± 5	16 ± 3	27 ± 4
8	67 ± 6	10 ± 3	23 ± 5	67 ± 5	7 ± 2	26 ± 5	12	58 ± 4	13 ± 2	29 ± 2	56 ± 3	13 ± 2	31 ± 3
10	68 ± 6	8 ± 2	24 ± 3	70 ± 5	6 ± 3	24 ± 4	16	59 ± 3	12 ± 1	29 ± 5	58 ± 4	11 ± 2	31 ± 2
							20	60 ± 5	12 ± 2	28 ± 3	59 ± 2	11 ± 1	30 ± 4
							24	63 ± 2	10 ± 2	27 ± 2	62 ± 4	10 ± 3	28 ± 3

**Table 2 Muscle fiber cross-sectional area of soleus of rats and rabbits at postnatal development**  
(Mean  $\pm$  SD,  $\mu\text{m}^2$ )

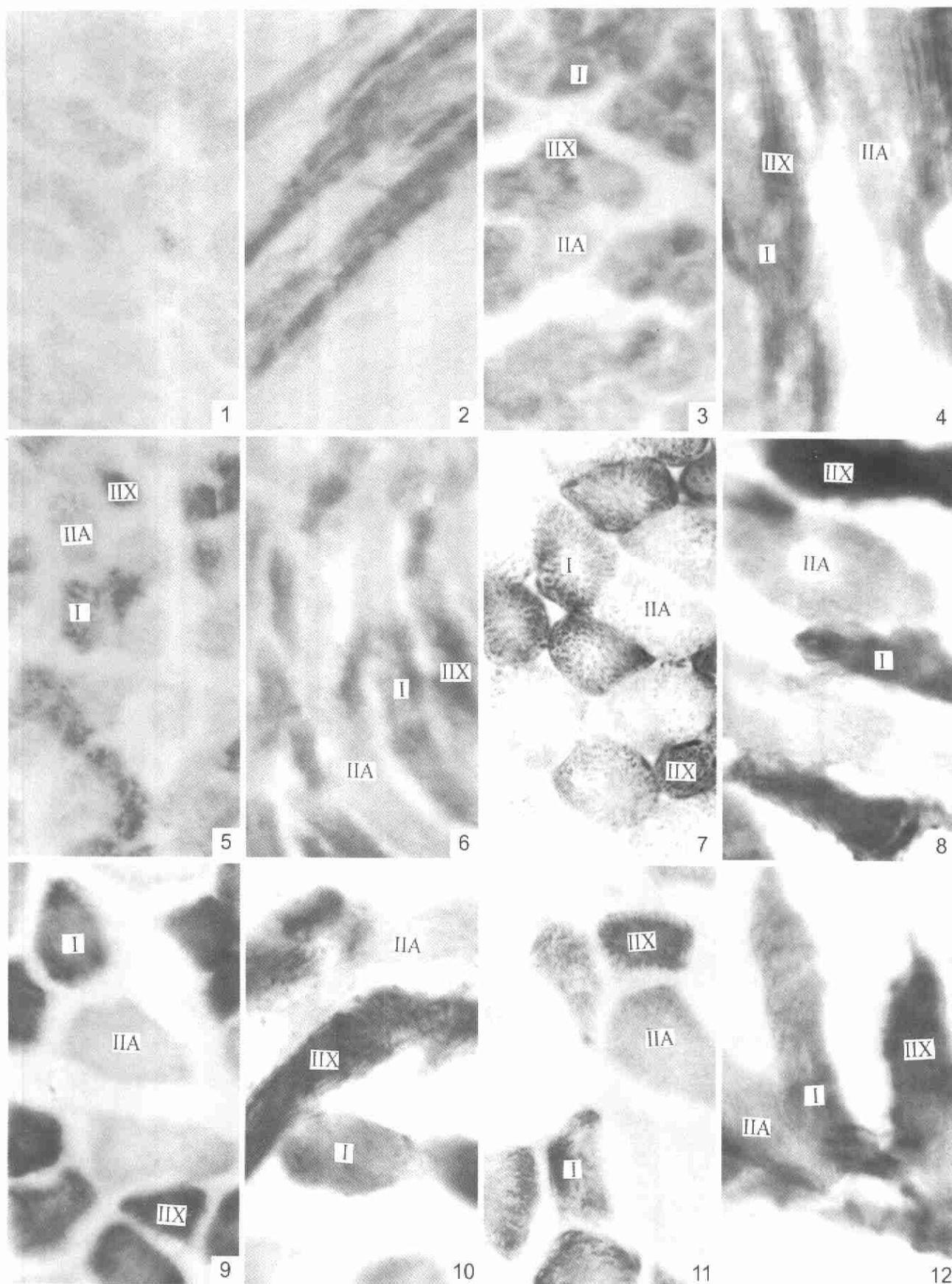
Age (week)	Rat					
	Left side			Right side		
	I	II X	II A	I	II X	II A
2 (d)	0	0	0	0	0	0
2	192 $\pm$ 31	61 $\pm$ 16	347 $\pm$ 47	192 $\pm$ 29	73 $\pm$ 21	391 $\pm$ 41
4	247 $\pm$ 29	104 $\pm$ 25	525 $\pm$ 21	265 $\pm$ 32	134 $\pm$ 19	595 $\pm$ 38
6	425 $\pm$ 42	198 $\pm$ 29	1 074 $\pm$ 45	523 $\pm$ 36	235 $\pm$ 33	1 168 $\pm$ 35
8	795 $\pm$ 38	289 $\pm$ 37	1 432 $\pm$ 36	770 $\pm$ 45	310 $\pm$ 42	1 668 $\pm$ 37
10	1 138 $\pm$ 35	476 $\pm$ 35	2 217 $\pm$ 39	1 039 $\pm$ 18	498 $\pm$ 37	2 410 $\pm$ 26
Age (week)	Rabbit					
	Left side			Right side		
	I	II X	II A	I	II X	II A
2 (d)	0	0	0	0	0	0
2	201 $\pm$ 31	134 $\pm$ 26	395 $\pm$ 35	195 $\pm$ 30	145 $\pm$ 23	357 $\pm$ 33
4	433 $\pm$ 40	191 $\pm$ 25	635 $\pm$ 42	384 $\pm$ 41	205 $\pm$ 18	595 $\pm$ 45
8	725 $\pm$ 37	387 $\pm$ 32	1 147 $\pm$ 28	697 $\pm$ 19	399 $\pm$ 26	1 087 $\pm$ 38
12	925 $\pm$ 31	503 $\pm$ 34	1 428 $\pm$ 46	993 $\pm$ 38	554 $\pm$ 29	1 440 $\pm$ 35
16	1 188 $\pm$ 29	698 $\pm$ 27	1 821 $\pm$ 43	1 202 $\pm$ 37	727 $\pm$ 32	1 903 $\pm$ 26
20	1 508 $\pm$ 41	924 $\pm$ 32	2 425 $\pm$ 45	1 549 $\pm$ 43	971 $\pm$ 41	2 525 $\pm$ 48
24	1 763 $\pm$ 47	1 138 $\pm$ 37	2 709 $\pm$ 41	1 803 $\pm$ 46	1 157 $\pm$ 39	2 810 $\pm$ 39

### 3 Discussion

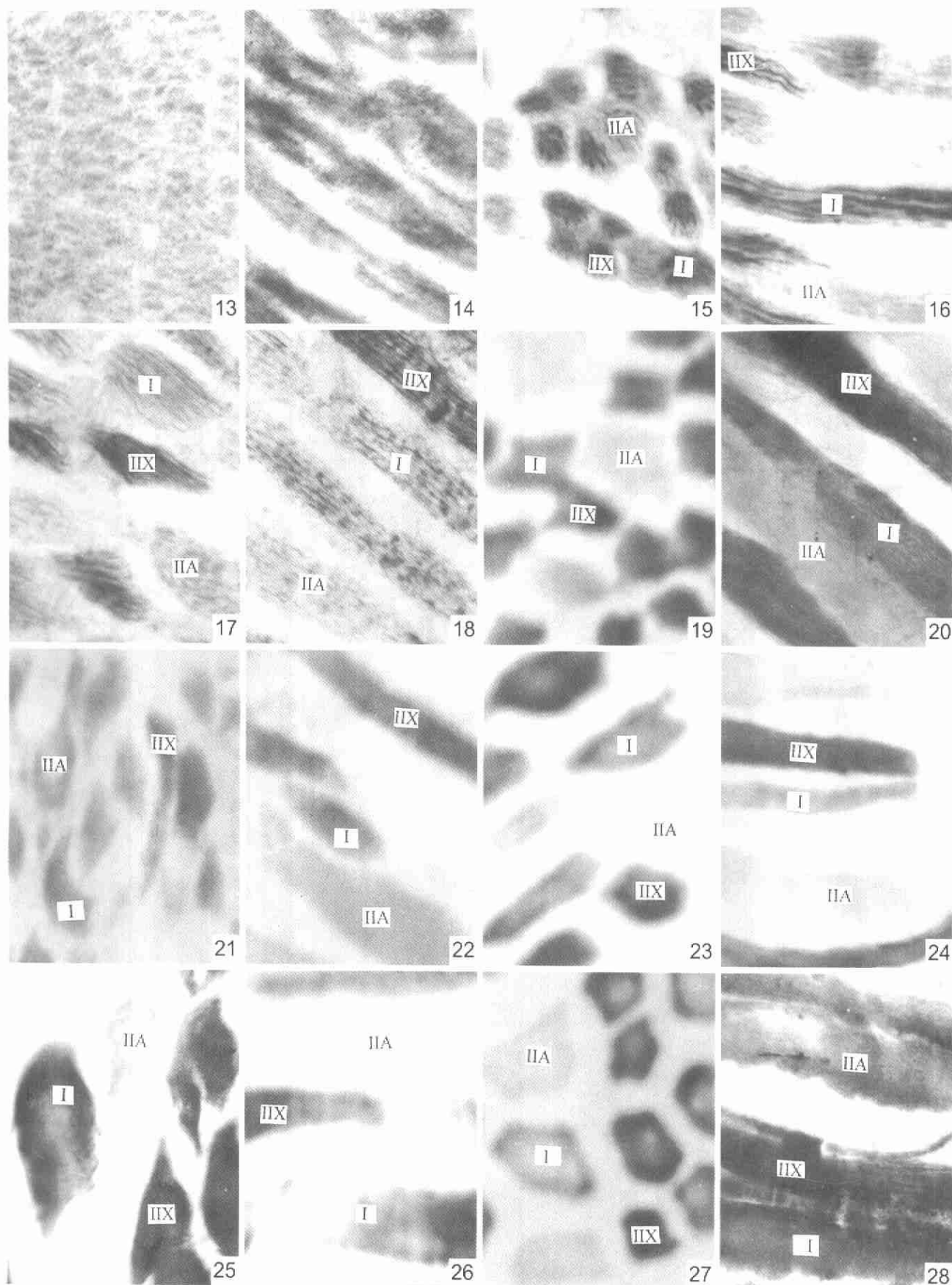
In the present study, the fibers of skeletal muscles in rats and rabbits were classified into three major types I, II X and II A on the basis of the histochemical staining patterns. Type II X fibers were difficult to distinguish from type I fibers even stained with mATPase under different pH values during preincubation (Denardi et al, 1993; Pette & Staron, 2001). Brooke & Kaiser (1970) found type II C fibers stained with mATPase, which were identified as a transitional type between I and II A. More recently, in addition to type I, II X, II A and II B, other transitional types such as I/II X, II X/II A and II A/II B were co-expressed by the corresponding myosin heavy chain (MyHC) isoforms (Klitgaard et al, 1990). Several consecutive sections are needed to identify each muscle fiber type with different MyHC isoforms. For the purpose of analysing the spatial distribution of muscle fibers in the X-Y coordinate, three fiber types or four fiber types should be identified in the same slide. The modified method used in the present study allowed identification of three fiber types after stained with SDHase.

Most types of muscle fibers in animals showed regional variations in distribution. The results of present study showed that type I and II X fibers located at the whole cross-section of the muscle, while type II A fibers were mainly at the central and deep regions during childhood. Both the number and distribution of

type II A fibers showed a little or no change with aging. Acosta & Roy (1987) suggested that a high percentage of type I was found in the deep region compared to the superficial region. The result of present study disagreed with Acostas' suggestion but well agreed with those of other workers (Fuentes et al, 1998; Zhu, 2003a, b). Two regions within the same muscle showed different percentages of fibers of different types. For fulfilling two different motilities in one muscle, the intertendons, nerves and vessels are regionally segregated to the superficial and deep regions with aging. Maintenance of posture is accomplished by the steady contraction of type I fibers located at the deeper region of the muscle. These fibers are more fatigue-resistant and less powerful than the fast type fibers. When the muscle performs faster and stronger contractions beyond the limits of type I, fast muscle fibers such as type II X (FO), II A (FOG) and II B (FG) might be subsequently recruited. Muscle contraction might propagate within the same muscle from the deep to the superficial regions. Type I fibers near a joint might contract for articular control, and these contraction extend to the distal region for further control of articular endurance. In this regard, previous studies have demonstrated that the number of type I fibers in the deep region may contribute to postural control and articular endurance, while type II fibers could be related to power and speed of movement (Gil et al, 2001). Our experimental re-



Figs. 1 - 12 Cross-section and vertical-section of muscle fibers of the rat soleus muscles at postnatal 2 days (1, 2), 2 weeks (3, 4), 4 weeks (5, 6), 6 weeks (7, 8), 8 weeks (9, 10) and 10 weeks (11, 12) ages, stained with SDHase.  $\times 200 - 400$



Figs. 13 - 28 Cross-section and vertical-section of muscle fibers of the rabbit soleus muscles at postnatal 2 days (13, 14), 2 weeks (15, 16), 4 weeks (17, 18), 8 weeks (19, 20), 12 weeks (21, 22), 16 weeks (23, 24), 20 weeks (25, 26), 24 weeks (27, 28) ages stained with SDHase.  $\times 200 - 400$

sults sustain this hypothesis.

The soleus muscle is frequently considered unusual in its fiber composition, metabolic and contractile properties (Maltin et al, 1989; Andruchov et al, 2003). The muscle is situated deep the medial and lateral heads of the gastrocnemius muscle and together they constitute the triceps surae. The function of the triceps surae is flexion of the knee and extension of the ankle joints. Considering the triceps surae as one unit, the soleus muscle presents the deep portion of these muscles. The high proportion of type I in the soleus muscle changes more with growth whereas no II B is observed, a mixed highly oxidative muscle. This is in accordance with previous results showing no II B expression in the deep red portion of rats and rabbits soleus muscle (Pellegrino et al, 2003). In the soleus and the plantaris muscles, type I fibers were homogeneously distributed throughout the cross-sections of the muscles. Both of the muscles constitute the deep compartment of the triceps surae and the stronger flexor muscles of the lower leg. In large quadrupeds such as horses and cattle, limb muscles may control posture allowing suspension of body weight during sleep. Their muscle fiber composition changes less by aging and exercise.

Type I fiber in rat and rabbit skeletal muscles are generally more sensitive to  $\text{Ca}^{2+}$  than type II fibers (Mounier et al, 1989). Myofibrillar  $\text{Ca}^{2+}$  sensitivity is determined mainly by the regulatory proteins tropomyosin and troponin (Piquet et al, 1997). Moreover, higher  $\text{Ca}^{2+}$  sensitivity can be caused by a higher ratio between the rates of myosin head attachment and force generation versus detachment to/from the actin filament. In the present study, type I fibers were associated with higher  $\text{Ca}^{2+}$  sensitivity, which was in accordance with predictions. Thus, the different kinetics of two slow MHC (muscle heavy chain) isoforms could be responsible for the differences in  $\text{Ca}^{2+}$  sensitivity of type I fibers in soleus muscles of rat and rabbit. Furthermore, postnatal translational modification of myofibrillar proteins must be considered as a possible modifying factor for calcium sensitivity of force generation. As to spatial distribution, muscle

fibers might be scattered randomly or dispersed homogeneously during the prenatal period. Differentiation to adult fiber type from neonatal fibers without innervation might be intrinsic to the muscle. In adult animals, the nerve innervating the muscle may play an important role in the maintenance and plasticity of fiber type-specific profiles (Pette & Staron, 2001). This has been demonstrated in studies examining the effects of cross-reinnervation. The specific function in each muscle might be dependent on the regional variation. Depending on the number and diameter of nerve fibers, and the quantity of hormone and blood supply, the distribution of fiber type in muscle might change from the homogeneous to heterogeneous during development.

In the present study, the soleus muscles of rats and rabbits was found as for the diameter of muscle fibers, type II A > I > II X. It was in accordance with others studies on the soleus muscle (Mattson et al, 2002). The fact that type II X fibers were the smallest can therefore be considered as a normal phenomenon in the soleus muscle. Some investigators (Maltin et al, 1989; Pellegrino et al, 2003) observed that, in contrast to what was normally found in skeletal muscles, type II X fibers were also smaller than type I fibers in leg skeletal muscles of rat and rabbit. A reduction in fiber size of type II X fibers in skeletal muscles was said to be associated with their histochemical characteristics and also with the oxidative activity. Table 2 showed that both the intra and interindividual variability of the cross-sectional areas of type II A fibers and type I fibers was relatively slight, while that of type II X and hybrid fibers was relatively obvious. This suggests that II X and hybrid fiber types are more prone to changes than type I fibers. Moreover, Table 2 showed that mean cross-sectional area of fibers of each type in rabbits was larger than that in rats. It was suggested that the size of muscle fibers is related to the animal species.

**Acknowledgement:** We are grateful to Prof. CHEN Jian-xiu in College of Life Sciences, Nanjing University for elaborately revising of the manuscript.

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